II. REMARKS AND ARGUMENTS

A. Remarks regarding the amendments

Claims 32-38 and 44-50 are pending in the application. Misnumbered claims 43-47 (added by amendment filed 4/11/03) have been renumbered 44-48. Claim 48 is amended to change its dependency from claim 46 to claim 47.

New claims 49 and 50 have been added. Example 3 of the specification supports these claims.

B. Claim rejections under 35 U.S.C. § 112

Claims 32-38 and 44-48 were rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. Specifically, the Examiner alleges that the definition "exogenous sensing moiety" does not clearly distinguish the sensing moiety from all types of sensing moieties that specifically bind to an analyte. Applicants respectfully traverse.

First, Applicants respectfully clarify that the term exogenous does not define a particular species of sensing moiety, but rather, defines how the sensing moiety is attached to the pore-subunit. The term exogenous indicates that the sensing moiety is covalently attached to the polypeptide. *See*, p. 3, ll. 10-18. The term exogenous differentiates the modified polypeptide subunits of the present invention, from polypeptides wherein the sensing moiety is engineered solely by mutations within the amino acid sequence of the polypeptide itself, i.e., wherein the sensing moiety is endogenous.

Written description for the term "sensing moiety" is provided by the specification at p. 7, l. 13-p. 8, l. 7. In various embodiments, the sensing moiety can be either a functional group or a polymer. Examples of sensing moieties that are specifically described include enzyme inhibitors, haptens, nucleotides, amino acids, lipids, toxins, saccharides, chelators and/or

cyclodextrins, calixarenes and/or crown ethers, homopolymers, heteropolymers, functionalized polymers, polyethylene glycol (PEG) or polyethylene glycol (PEG)-biotin, oligonucleotides, polynucleotides, oligosaccharides, polysaccharides, lipopolysaccharides, proteins, glycoproteins, polypeptides and/or peptides. In particularly preferred embodiments, the attached polymer is a single-stranded oligonucleotide or polynucleotide, such as DNA or RNA.

The Examiner contends that example 3 is drawn to an oligonucleotide "exogenous sensing moiety" with a specific sequence and does not provide an adequate representation of all types of sensing moieties. The Examiner is ignoring that the specification provides written description of many sensing moieties other than the specific oligonucleotide of example 3 and that one of skill in the art can appreciate many additional sensing moieties, in light of the present disclosure. The scope of the claims is not limited by the examples.

The Examiner cited *University of California v. Eli Lilly and Co.*, as suggesting that the written description requirement would only be met if the specification contains a structure of formula for all types of exogenous sensing moieties. Contrary to the Examiner's assertion that the holding of *Eli Lilly* would be applicable to any compound, the Federal Circuit stated in *Amgen, Inc. v. Hoecht Marion Roussel, Inc.* that *Eli Lilly* is inapposite to claim terms that are "not new or unknown biological materials that ordinarily skilled artisans would easily miscomprehend." 314 F.3d 1313, 1332 (Fed. Cir. 2003). The claim term at issue here is "sensing moiety capable of preferentially binding with a specific analyte." Such sensing moieties are well known in the art. For example, it is well known in the art to use an oligonucleotide to bind, and thereby sense, complimentary analyte DNA. Likewise, a variety of crown ethers are available that preferentially bind various metal ions. One of skill in the art is

familiar with this concept and would not miscomprehend that various sensing moieties preferentially bind with specific analytes.

The court also stated in *Amgen, Inc.*, "*Eli Lilly*, did not hold that all functional descriptions of genetic material necessarily fail as a matter of law to meet the written description requirement; rather, the requirement may be satisfied if in the knowledge of the art the disclosed function is sufficiently correlated to a particular, known structure." *Amgen, Inc. v. Hoecht Marion Roussel, Inc.* 314 F.3d 1313, 1332 (Fed. Cir. 2003). As described above, the functional language "capable of binding with a specific analyte" is sufficiently correlated with known structures because the art is replete with known structures for binding particular analytes.

In summary, (1) "exogenous" is clearly defined in the specification as meaning that the sensing moiety is covalently bound to the modified pore subunit, as opposed to arising solely due to mutations within the amino acid sequence of the polypeptide itself; (2) that additional sensing moieties, other than the particular sensing moiety of example 3, are specifically described in the specification; and (3) one of skill in the art would appreciate many additional known structures for binding specific analytes based on the functional language of the claims. Applicants submit that the specification meats the written description requirement for the entire scope of claim 32 and respectfully request that the rejection under 35 U.S.C. § 112, first paragraph, be withdrawn.

C. Rejections under35 U.S.C. § 102

1. The Church reference.

Claims 32-38 and 44-47 have been rejected under 35 U.S.C. § 102(b) as being anticipated by U.S. Pat. No. 5,795,782, by Church et al. (the Church reference). Specifically, the Examiner alleges that Church discloses a method of detecting an individual polymer molecule by an interface, which comprises an ion permeable passage wherein the ionic conductance of the

passage changes as each monomer of the polymer interacts. The passage is either a protein channel or a recombinant bacterial porin molecule. The Examiner further alleges that the protein channel assembles by covalent linkage by expressed protein, the polymer to be characterized includes a portion that acts as a specific ligand for the receptor, that electrical current can be detected through a single channel or a two channel system, that the method can identify the individual monomers in the polymer, that the polymer is any biological polymer, and that the concentration of the polymer can be determined. The Examiner concludes that Church anticipates the claimed invention. Applicants respectfully traverse.

The Church reference describes different embodiments of methods and structures for characterizing linear polymer molecules by measuring physical changes across an interface between two pools of media as the linear polymer traverses the interface and monomers of the polymer interact with the interface. The methods of Church are directed to characterizing the size or sequence of polymers. See, col. 1, ll. 35-39. These embodiments are briefly described below.

Bacteriophage receptor. This embodiment is described in the introduction of Church at col. 3, ll. 28-36 and in the description at col. 13, l. 55 - col. 15, l. 5. According to this embodiment, a protein that includes a portion of a bacteriophage receptor is positioned at an interface. The bacteriophage receptor is capable of binding all or part of a bacteriophage ligand. The polymer to be characterized includes a portion of the specific ligand for the bacteriophage receptor so that it may be injected across the interface. The polymer is characterized as it passes through the pore. The sequence of steps for such an analysis is described in Example 1, beginning at col. 14, l. 52:

> The conductance of single LamB pores is monitored during the addition of phage to the medium bathing the bilayer. An initial change in conductance upon phage binding will be followed by a drop in conductance as DNA enters the pore. Any sustained conductance fluctuations that follow are indicative of base pairs passing through the pore during injection. The fluctuations should be in the millisecond range, and the period of fluctuation will generally last for about 60 sec (the time required for injection). The conductance should then go up again to a level even higher than the original pre-phage state, since post-injection phage/porin complexes have been observed to allow molecules larger than the normal LamB exclusion limit to pass through (Roessner et al., 1986, J. Biol. Chem., 261:386-90).
>
> Asymmetrically modified DNA produced by annealing modified and unmodified complementary strands or by custom primed DNA synthesis, can be ligated to lambda vector DNA and packaged in vitro. Modified DNA that is packaged efficiently and can be injected into bacterial cells will be appropriate for the LamB sequencing system.

This explanation clarifies that the Church method is not directed to detecting the presence of a specific analyte, rather, it is directed to characterizing, i.e., sequencing, a polymer molecule. In other words, the Church device does not screen to see if a particular DNA sequence is present; it sequences whatever DNA is there.

The Examiner alleges that the sentence "The polymer to be characterized includes a portion which acts as a specific ligand for the bateriophage receptor, so that it may be injected across the barrier/interface from one pool to the other . . ." anticipates the claim element "exogenous sensing moiety capable of preferentially binding with a specific analyte." However, when the Church reference is taken as a whole, as it must be, it is clear that the "specific ligand" that is contained on the "polymer to characterized" is not a specific analyte. All of the polymers to be characterized will contain this ligand. The Church method does not determine if this ligand is present because it is known ahead of time that the ligand is present. The receptor of Church will bind <u>whatever</u> phage ligand is present; this binding is simply the method of getting whatever

DNA is present in the medium to interact with the pore so that it can be sequenced, i.e., characterized.

In contrast, the instant claims recite a sensing moiety capable of preferentially binding a specific analyte. This binding, as measured by a modulation in current through the pore, determines whether or not the specific analyte is present in the sample. The instant method allows one to screen for a particular analyte without sequencing every analyte that is present.

Further, the Church reference does not indicate that the phage-binding receptor is exogenously attached to the pore in this embodiment. Rather, the phage-binding receptor is part of the native pore or mutated pore. *See*, col. 13, l. 55-col. 14, l. 35.

Pore bound to a polymerase molecule to pass DNA over the pore's opening. An alternative embodiment described by Church is a pore that is fused to a polymerase molecule. This embodiment is depicted in Fig. 2 and is described at col. 3, l. 38 - col. 4, l. 30 and col. 15, ll. 5-64. According to this embodiment, the polymerase draws whatever polymer is present in the medium across the opening of the pore as it synthesizes a new polymer from the template polymer. The polymer is sequenced as it is drawn across the mouth of the pore.

As with the previous embodiment, this polymerase molecule does not preferentially bind any one specific analyte molecule, rather, it will draw whatever DNA is present in the medium across the mouth of the pore. The purpose of the polymerase is not to bind a specific analyte; it is to move whatever analyte is present across the opening of the pore so it can be sequenced.

In formulating her rejection, the Examiner has switched back and forth between these two embodiments to derive the elements that she alleges anticipates the instant claims. As clarified above, there is no evidence that the bacteriophage receptor is exogenous. Rather it is taught that the bacteriophage receptor is a native or mutant protein. On the other hand, to the extent that the

polymerase molecule of the alternative embodiment is covalently attached to the pore, it may in fact be exogenous, as defined in the present disclosure. However, this polymerase molecule does not bind a specific analyte, rather, it will interact with any template polymer that is solution. There is not an embodiment described in the Church reference that teaches an exogenous sensing moiety capable of preferentially binding a specific analyte.

In summary, the Church reference is not directed to methods of detecting the presence of specific analytes, rather, it is directed to methods of characterizing, i.e., sequencing polymer molecules. As such, the devices of Church do not comprise a sensing moiety that preferentially binds with a specific analytes. Rather, they are designed to bind to all molecules that are present and move the molecules across the opening of the pore so that they can be sequenced. It would be contrary to the operation of the Church device if the device could only bind a single, specific analyte, because the device would no longer be capable of sequencing various polymer molecules.

The present claims are directed to a method of detecting a specific analyte, i.e., a method of screening for the presence of a known analyte. The present invention therefore comprises a sensing moiety capable of preferentially binding a specific analyte.

Applicants respectfully request that the rejection over the Church reference be withdrawn because the Church reference does not teach a method of detecting the presence of a specific analyte in a sample or an exogenous sensing moiety capable of preferentially binding a specific analyte.

The Church reference does n t teach an oligonuleotide as a sensing moiety. Applicants note that claims 44-47 were rejected under 35 U.S.C. § 102 over the Church reference, but that the Examiner did not make any attempt to specifically apply Church to these

claims. For example, the Examiner did not explain where the Church reference teaches an exogenous sensing moiety that is an oligonucleotide or a polynucleotide, as is recited in claims 45 and 47, or an exogenous sensing moiety that is single stranded DNA, as is recited in claim 46. Applicants respectfully request that this rejection be withdrawn.

2. The Braha reference

Claims 32-33, 35 and 38 were rejected under 35 U.S.C. 102(b) as being anticipated by Braha et al., *Chemistry & Biology*, 4(7): 497-505, 1997 (the Braha reference). Specifically, the Examiner alleges that the Braha reference anticipates the instant claims because it discloses a method of detecting divalent metal ions using a bacterial pore-forming protein having receptor sites. The Examiner notes that the 4H subunit was tagged by chemical modification of a single cysteine with 4-acetamido-4'[(iodoacetyl)amino]stilbene-2-2'-disulfonate (IASD). The Examner alleges that the IASD is an exogenous sensing moiety. Applicants respectfully traverse.

The Braha reference is directed to a biosensing architecture utilizing an α-hemolysin, in which pore-subunits have been engineered to contain a binding cite for a divalent metal ion. *See*, Braha reference, abstract and Figure 1. The only modifications to the pore-subunit polypeptides disclosed in the Braha reference that are relevant to the sensing mechanism are mutations within the amino acid sequence of the polypeptide itself, i.e., the peptides comprise only an "endogenous" sensing moiety.

Contrary to the Examiner's assertion, the IASD is not a sensing moiety at all, rather, it is simply attached to the pore subunit to increase the subunit's electropohoretic mobility in SDS-polyacrylamide gel so that the mutated pore subunit can be isolated from the wild type heptamers. *See*, Braha, page 499. The IASD has nothing to do with sensing analyte; it is distant

from the channel, which is the site of the endogenous sensing moiety. *See*, id. and Figure 1. The Braha reference does not teach an exogenous sensing moiety.

In contrast, the instant claims are directed to a method of detecting an analyte using a pore assembly wherein at least one of the pore-subunit polypeptides is modified to contain an "exogenous" sensing moiety. Applicants therefore respectfully request that the rejection over the Braha reference be withdrawn.

D. Rejections under 35 U.S.C. § 103

Claims 32-38 and 44-48 have been rejected under 35 U.S.C. § 103(a) as being obvious over Braha, in view of Church. Specifically, the Examiner alleges Braha discloses a method of detecting divalent metal ions using a bacterial pore-forming protein having receptor sites. The Examiner acknowledges that Braha does not teach a sensing moiety that is an oligonucleotide. The Examiner alleges that Church teaches an exogenous sensing moiety that is an oligonucleotide and that it would have been obvious to include such an exogenous sensing moiety in the method of Braha. Applicants traverse.

Church does not teach an oligonucleotide as an exogenous sensing moiety or as a part of the sensing device. As described above, one embodiment of the Church invention comprises a polymerase enzyme. An alternative embodiment comprises a bacteriophage receptor. Neither of these moieties are oligonucleotides. Applicants are unclear how the Examiner arrives at the conclusion that Church teaches an oligonucleotide as a sensing moiety.

It was also pointed out above that Church does not teach any sensing moiety that preferentially binds a specific analyte. The Church devices are instead designed to characterize any polymer that might be present in solution. Therefore, the polymerase moiety or the bacteriophage receptor moiety of the Church devices are not provided to bind one specific

analyte, rather, they are provided to move any polymer that is present in solution across the pore opening so that it can be sequenced. The Church reference does not provide an exogenous sensing moiety capable of preferentially binding a specific analyte.

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The Examiner is invited to contact the undersigned patent agent at 713-787-1558 with any comments relating to the referenced patent application.

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